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THE COMPOUND OOSPHERE OF ALBUGO BLITI

A DISSERTATION SUBMITTED TO THE FACULTIES OF THE GRADUATE
SCHOOLS OF ARTS, LITERATURE, AND SCIENCE, IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

BY

FRANK LINCOLN STEVENS

CHICAGO

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THE COMPOUND OOSPHERE OF ALBUGO BLITI.

THE results of this investigation introduce factors entirely new in their essential features and at variance with the cytological processes connected with fertilization previously described for coenocytic or other forms of plants or animals. The title of the paper must suggest a contradiction of terms. The writer hesitated before using the expression compound oosphere, but it seemed better, at least for the present, to modify the term oosphere which has become firmly fixed in descriptive botany so that it would express the peculiar conditions here set forth. If future investigations should indicate that the peculiarities of the compound oosphere are not exceptional, it may be necessary to introduce a new term indicating the conditions and the process of fertilization presented in this paper.

A compound oosphere is one containing several or many functional sexual nuclei. This idea violates the present conception of the structure of that cell as it exists throughout the plant kingdom, and furthermore, so far as the writer is able to judge, the ova of animals present no such peculiarity. If there is any character that defines the oosphere and ovum it appears to be the presence in the cytoplasm of a single female nucleus that is normally fertilized by a single male nucleus. In *Albugo Bliti* Biv., however, the mature oosphere contains many female nuclei, and fertilization is effected by the discharge of many male nuclei from the antheridial tube and their subsequent fusion with the female nuclei in pairs. An oospore results from this multiple sexual act with about one hundred fusion nuclei, which remain in the

resting condition until germination. The existence of such conditions must be supported by strong evidence, and great caution should be exercised in interpreting the data upon which the conclusions are based.

One is partially prepared, however, for the acceptance of such conditions as these by the thought that the form under consideration is a coenocyte, and that comparatively little is known of the behavior of the nuclei and cytoplasm in such structures. Excellent summaries of current knowledge are given by Humphrey ('92), Zimmerman ('96), and Wager ('96). It is unnecessary, in view of the existence of these accounts, to enter into details here. Suffice it to say that in several (Monoblepharidaceæ, Entomophthoraceæ, and Chytridineæ) of the many coenocytic groups the behavior of the nuclei in fertilization is practically unknown. In those groups of which there is more knowledge (Saprolegniaceæ, Peronosporæ, Zygomycetes, and Siphonæ) concordant results have not yet been attained. For example, in the Saprolegniaceæ the question is still in dispute whether or not fertilization occurs (Hartog '95, Trow '95). In the Siphonæ the two most comprehensive papers (Behrens '90, Oltmanns '95) upon the one genus (*Vaucheria*) that has been investigated disagree essentially as to the events leading to the development of the oosphere. The process of fertilization described for the Zygomycetes by Léger ('95) involves the unique phenomenon of the fusion of nuclear complexes.

The pioneer work on the histology of the Peronosporæ, done by Wager on *Peronospora parasitica* in 1890, was followed by an article by the same author in 1896 on *Albugo candidus*. Berlese in 1898 published an article on the entire group. It appears that most of the study of the histology of coenocytic fungi has been concerned with the Peronosporæ, but even here the data are yet too scanty to admit of any wide generalizations. The improvements in technique during recent years should be held in mind, as much of the discrepancy between the earlier and later results may be thus explained.

Wager's work ('96) should be consulted for a comprehensive

summary of the knowledge up to the time when he published the results of his investigations. For *Albugo candidus* his own research shows a condition where the antheridial tube liberates one sperm nucleus which fuses with the solitary female nucleus in the ooplasm. He employed corrosive sublimate in saturated aqueous solution as a fixing agent, and stained the sections with Hartog's nigrosin-carmin. In his earlier work on *Peronospora* Wager describes a multinucleate oogonium and antheridium. The nuclei of the maturing oogonium pass to the periphery, where they divide mitotically. Two or three then return to the center and probably fuse, as only one nucleus is found there at a later stage. The antheridial nuclei divide simultaneously with those of the oogonium. The antheridial tube contains one or more nuclei, the antheridium finally having many less than it had at an earlier stage. This material was fixed by either absolute alcohol or chromic acid, and stained with Kleinenberg's hæmatoxylin.

Berlese gives four figures to illustrate the development and fertilization of *A. Portulacæ*, but his description of the nuclear transformation is not illustrated. All statements concerning *Albugo* seem to have been based on either this species or herbarium specimens of other species. He studied also four species of *Peronospora*. For a killing agent he used either 95 per cent. alcohol, alcoholic corrosive sublimate, Flemming's solution, or picric acid, and stained with Flemming's triple stain or Hartog's nigrosin-carmin. Further reference will be made to these articles later in the paper.

The results presented in this paper were obtained mainly from material fixed in chrom-acetic acid, cut in serial sections in paraffin, and stained on the slide by Flemming's triple stain. For full details regarding methods the reader is referred to the end of the paper. This investigation was begun in 1897, one year being spent in the botanical laboratory of the Ohio State University through the kindness of Dr. W. A. Kellerman. I am indebted to Mr. J. H. Schaffner for many courtesies during my work at the same institution. Since the summer of 1898 the study has been continued under the direction of Dr. Bradley

Moore Davis in the Hull Botanical laboratory of the University of Chicago, where I have also received helpful advice and suggestions from Dr. J. M. Coulter and the members of the botanical staff. I wish to express my thanks to my wife for much kind assistance, in particular for the preparation of Plate XV.

DEVELOPMENT OF THE OOGONIUM AND ANTHERIDIUM.

The character of the mycelium of *Albugo* varies with the nature of the host tissue. The hyphae are slender where the cells of the host are thick-walled and placed close together, while in loose tissue they may swell to a considerable diameter. *Fig. 42* shows the general structure of the hyphae, the roundish nuclei, each with a prominent nucleolus and membrane, being distributed irregularly through the vacuolate cytoplasm. A single nucleus is represented in *fig. 1*. It is worthy of attention principally because of its very faint linin network. It has an actual diameter of from 2–2.5 μ .

The oogonium may be terminal or intercalary, its walls being simply the greatly expanded mycelial wall, as is evidenced by the frequent persistence of haustoria on its surface. Even very early stages of developing oogonia may be distinguished from enlarged mycelia by certain peculiarities of the protoplasm (*fig. 43*.) The nuclei are elongated, the vacuoles are angular and distorted, and the cytoplasm is drawn into stringy bands; all of which gives evidence of a disturbance not present in the ordinary mycelium. These peculiarities are frequently evident in vegetative hyphae a distance of 200 μ from the developing oogonium. Similar appearances were noted by Istvanffi ('95) and Wager ('96), and their explanation is undoubtedly the true one, namely, that the protoplasm was rapidly flowing from the mycelium to fill the enlarging oogonium. When sufficient nucleated protoplasm has entered the developing oogonium, this structure is cut off from the hyphae by a septum at the point of enlargement (*fig. 44*). The oogonium is now fully differentiated from the vegetative hyphae, the nuclei recover their original form and lie in a coarsely vacuolate and somewhat granular cytoplasm. The

general appearance of the oogonium and its contents may be seen in *fig. 45*.

The antheridium develops simultaneously with the oogonium, but gives no evidence of the flowing of the protoplasm into the growing structure. Probably owing to the small size of this organ there is but little disturbance as it fills with protoplasm. It becomes cut off from the parent hypha and the contents are similar in appearance to those of the oogonium as is shown in *fig. 45*.

The most conspicuous feature in this early development of the oogonium is the increase in the size of the nuclei. This seems to occur somewhat rapidly just before the oogonium has attained its full size. As the nuclei grow larger the linin network becomes much more prominent, until finally it assumes a very characteristic structure in the form of large meshes, the threads being coarse, densely staining, and apparently homogeneous in structure. The whole appears to be a connected network that lies against the nuclear membrane. When the threads are seen in transverse view they appear as round or oblong bodies about the size of the nucleoli, but staining differently. This condition of the nucleus, which is shown in *fig. 3*, may be regarded as the spirem stage of early mitosis. While the nuclei are passing into this condition the oogonium wall thickens slightly.

It is at this time that the number of nuclei may be determined most easily and accurately. The count ranged from 226 to 333, the smaller number being quite exceptional. Making allowance for the fact that several nuclei may readily have been counted twice in adjacent sections, it would perhaps be fair to place the average at about 250. Wager found 115 in *A. candidus*, and Berlese 200 in *A. Portulacæ*. It is very difficult to make an accurate count in the antheridium, because this structure is so small and of such irregular shape that it is impossible to recognize its limits in adjacent sections. However, an average based on several counts indicates that the number is likely to be about 35. This number is considerably greater than that suggested by either Wager or Berlese, both of whom report about 12 nuclei in the antheridium.

When the oogonial nuclei are passing into the spirem condition the *Hautschicht* seems to be closely appressed to the wall in the vicinity of the antheridium. This fact is demonstrated most clearly in preparations where there has been slight collapse of the contents of the oogonium (figs. 47, 48) and the protoplasm has shrunk away from the wall everywhere excepting at the point opposite the antheridium. This adherence of the *Hautschicht* is correlated with a very marked granulation of the cytoplasm in this region, a phenomenon also noted by Wager, and one which seems to be significant. It suggests that a cellulose enzyme is secreted to dissolve the wall of the oogonium. As indicated in figs. 48, 49, 50, this wall frequently shows the marks of corrosion over a considerable area, always at a point opposite the antheridium. This interesting process results in a neat perforation, through which the cytoplasm of the oogonium flows so as to form a very conspicuous swollen papilla within the antheridium. Various stages in this process are shown in figs. 50-55. It is difficult to explain this phenomenon. The initiatory step in the perforation of the oogonium wall seems without doubt to be taken by the protoplasm of the oogonium itself. But what is the significance of the pushing of the cytoplasm of the oogonium into the antheridium to form the peculiar bubble-like papilla? The structure, both wall and contents, stains deeply, thus becoming very conspicuous, while its extremely frequent occurrence, as well as its presence in other species, seems to indicate that it is not abnormal. The papilla wall is so extremely thin that one may only conjecture whether or not it is derived from the antheridium wall. The history of the papilla beyond the stage shown in fig. 54 is not clear. In this condition the structure is very thin-walled and its contents vacuolate, resembling a large irregular compound bubble. Whether the delicate wall now bursts or the contents are gradually withdrawn into the oogonium is uncertain. At all events, the papilla in later stages leaves no trace of its former existence. There follows at a later period conditions (figs. 56, 57) which show that there is certainly a movement of the cytoplasm in the opposite direction, the antheridium extend-

ing a process with a cell wall through the opening into the oogonium. The first surge of the cytoplasm from the oogonium into the antheridium may be due simply to the unequal conditions of turgor in the two structures, but it is possible that there is also a phylogenetic significance in the phenomenon. The occurrence of similar structures in *A. candidus* and *A. Portulacæ* shows it to be of some import. Such a papilla in a much less highly developed form is figured by Wager and referred to as the receptive papilla.

The antheridial tube presses into the oogonium in the form of a slender thin-walled process (*figs. 56, 57*). It is filled with dense cytoplasm that greedily absorbs and retains stain, and is surrounded by a sheath of dense oogonial cytoplasm. The nuclei remain in the antheridium, none entering the tube at this time, and are indistinguishable in size and structure from those in the oogonium. They are also in the spirem condition, similar to that described for the oogonial nuclei. The description of the further development of the antheridial tube is deferred, to follow the account of the differentiation of the compound oosphere.

DIFFERENTIATION OF THE COMPOUND OOSPHERE.

The previous description carries the history of the sex organs up to a time when the antheridial tube has penetrated the oogonium one fourth or one fifth the diameter of that structure. Correlated with the further development of the tube there occurs the differentiation of the periplasm and ooplasm, and the extrusion of the nuclei from the central region of the oogonium. The process consists essentially in a centripetal movement of the cytoplasm, and results in a massing of this cytoplasm in the center of the oogonium in such a manner that the vacuoles and nuclei are carried to the periphery of the denser central portion thus developed. Behrens ('90, 315) describes a somewhat similar condition in *Vaucheria* as follows: "Der ganze Vorgang besteht also in der Ablösung des grössten Theils der Protoplasten von der Wand durch Vacuolisation der wandständigen Plasmaschichten." This curious phenomenon was noted by Wager in *A. candidus*,

and subsequently by Berlese in *A. Portulacæ*. The process as heretofore described is simple. In *A. Bliti* it is complex, but unique and full of interest; and as a complete knowledge is essential to an understanding of the further development of the oosphere a detailed description must be given.

The first hint of the centripetal aggregation is found in a tendency of the cytoplasm to depart from the even distribution shown by a young oogonium, and to collect in masses throughout the interior (*fig. 58*). These denser portions run together, forming fewer but larger masses (*fig. 59*). Thus several prominent aggregations of cytoplasm may be formed, separated from one another and from the wall by vacuoles of varying sizes (*fig. 60*). These denser regions are homogeneous in structure, containing minute vacuoles of uniform size evenly distributed in a matrix of cytoplasm free from granules. The dense regions contain no nuclei, because these are forced from the dense cytoplasm to a position on its periphery. The dense centers now coalesce, forcing out the vacuoles. This may result immediately in the condition shown in *fig. 61*, but frequently the coalescence proceeds more slowly and irregularly, and often a reniform mass is formed, the indentation on one side marking the juncture yet to be made. The last gap narrows until only a few vacuoles remain to mark its track (*fig. 61*), and these soon float outward leaving one mass of cytoplasm, the rudimentary oosphere (*figs. 62, 64, 65*). As the vacuoles pass outward they often leave captive nuclei in their wake (*figs. 61, 62, 64*), but these soon follow. A typical view of the resulting condition is presented in *fig. 64*. The outer region of the oogonium, the rudimentary periplasm, is coarsely vacuolate, presenting a conspicuous contrast to the dense central mass. Along the boundary between these two regions are gathered most of the nuclei (*fig. 64*).

The next stage in the differentiation of the oosphere is conspicuous and clearly characterized. It ends in producing a distinct differentiation between the oosphere and the periplasm (*fig. 65*). This condition is brought about primarily by the marshaling of the nuclei into an oval or an irregular hollow sphere,

a section of which is shown in *fig. 65*, while a somewhat earlier stage is to be seen in *fig. 64*. Both figures illustrate the one important fact that all or nearly all of the nuclei are at the boundary of the central dense mass. The latter figure in addition shows that there is a sharp line of demarcation between the ooplasm and periplasm. There are usually a few scattered nuclei in the periplasm, and occasionally one finds a nucleus in the oosphere that has not passed out as rapidly as the others.

Important changes occur in the cytoplasm while the nuclei are arranging themselves into a hollow sphere. At the beginning of this process the region that is to become periplasm is coarsely vacuolate, in marked contrast to the dense cytoplasm of the center, but the two regions blend gradually together where they meet (*figs. 62, 64*). Later when the hollow sphere of nuclei becomes more regular in outline dense granular cytoplasm is differentiated around and between the nuclei (*figs. 65, 68, 69*). The inner border of the rudimentary periplasm also becomes differentiated into a film more densely granulated than any other region of the oogonium, and finally determines the limit of the oosphere. There is not yet an organized wall, and the most critical study reveals nothing more than a dense film of protoplasm. It is convenient to call this condition the stage of zonation.

The position of the stage of zonation in the sequence of events leading to the differentiation of the oosphere is clear. With this condition comes the characteristic and sharp limitation between ooplasm and periplasm which is maintained until maturity, while before zonation such a differentiation did not exist. The process of differentiation is gradual, and a series of developmental stages has been obtained which seems complete. This period is the only one where the ooplasm possesses very few nuclei or none, and it is impossible to regard it as being a period later than stages which present zonation and also contain 50-100 nuclei (*figs. 68, 69, 70*). The development of the antheridial tube is such as to lend strongest support to the sequence above indicated, since the tube is shorter in stages

preceding zonation and longer in stages following it (see plates), thus affording strong corroborative evidence. While the differentiating line is characteristic of zonation the paucity of nuclei in the ooplasm is equally so. The sharper the differentiation the fewer the nuclei, and when zonation was very definite none could be found, and it is probable that when this stage is at its highest development there are no nuclei in the ooplasm. There is some evidence, however, that makes it seem possible that one and even two spindles sometimes remain in the ooplasm, but this is uncertain.

No mention has been made, as yet, of the division of the nuclei of the oogonium. This mitosis closely accompanies the process of zonation as is indicated in most of the figures. These two events apparently take place nearly simultaneously. The earliest prophase is typical in such stages as are shown in *figs.* 58, 59, 60, 61. At the time of complete zonation the nuclei are in metaphase and lie close to the line that separates the ooplasm from the periplasm (*figs.* 65, 66). Spindles are frequently found that actually cross this line at right angles, so that one pole lies in the ooplasm and the other in the periplasm (*figs.* 65, 66). The mitoses that take place at this period mark an important and characteristic phase in the history of the oogonium. Those dividing nuclei that lie tangential to or wholly outside of the boundary line between the ooplasm and periplasm leave their daughter nuclei in the periplasm. Each of the spindles which cross the line (*fig.* 66) gives one daughter nucleus to the oosphere and the other to the periplasm, and the line of differentiation is sharply defined and unmistakable. Nuclei may be observed in every phase of this mitosis, and the daughter nuclei may be found in all stages of reorganization, one of each pair in the ooplasm the other in the periplasm. The writer was unable to detect any difference between the mitoses that occur strictly in the periplasm and those that contribute daughter nuclei to the oosphere. As a result of the division a large number of nuclei pass into the ooplasm, thus producing a multinucleate cell containing by actual count an average of 45 to 55 nuclei (not

less than 40 or more than 60). The oosphere is thus a coenocyte; instead of the uninucleate cell which one would expect there is found a multinucleate structure, to designate which the writer has used the term compound oosphere.

Because of the importance of the anomalous compound oosphere and the peculiarities of its development, it seems best to discuss at length difficulties that might be suggested. It may be claimed that a mistake has been made in the sequence of events, and that the multinucleate condition of the central region does not follow, but precedes zonation. This objection is invalid, for four reasons: (1) the sequence is complete up to zonation, and there is no place for a multinucleate central region in the series; (2) the studies in cytology make it certain that there is but one simultaneous mitosis of the oogonial nuclei, so that the phase of the division serves as an index of the age of the oogonium, thus rendering any misunderstanding of the sequence impossible; (3) the interpretation presented is necessary to the understanding of stages that are positively and unmistakably older; (4) the development of the antheridial tube and central body correlate with the views presented. It would seem impossible that a mistake in seriation has been made. It may be claimed that the daughter nuclei seen in the mature oosphere are not *in situ*, but were carried in by the knife. There is abundant evidence to controvert this claim, for the nuclei in anaphase often lie in such positions that, if carried in by the knife, they must have been carried in two opposite directions by the same stroke. It may be well to state that specimens of every structure or stage represented or described in this article are preserved, not in one, but in several mounts, and for most of the important stages in many preparations. No isolated or single fact is anywhere used either to support or destroy any theory.

With the entrance of the nuclei differentiation of the oosphere is complete. The ooplasm is of very fine, even texture, made up of such small meshes that the vacuoles are never more than half the size of the nuclei, and there are no prominent granules or oil drops shown by the Flemming stains at this time (*figs. 68-*

69). The periplasm is loosely vacuolate, the strands are often granular, and the nuclei are frequently at their intersections, often in bunches.

The nuclei of the antheridium usually divide simultaneously with those of the oogonium, this being so constantly true that from a glimpse of one organ the condition of the other could be predicted. No difference between mitosis in the oogonium and in the antheridium could be observed. At the time of the differentiation of the oosphere the contents of the antheridial tube stain deeply, but it has not been possible to demonstrate nuclei in its interior at this age. When the nuclei line up preparatory to zonation the antheridial tube has usually penetrated the periplasm almost to the outer boundary of the oosphere, and as it later pushes into the oosphere, during the telophase of the mitosis, it somewhat indents the boundary film. One oogonium was seen that had two antheridial tubes penetrating the periplasm from opposite sides, but neither had yet pierced the boundary of the oosphere. Another stage was observed where an unemptied tube lay tangential to the oosphere, being apparently the unfavored of two competitors. Still another case presented a stage of fertilization in which two tubes were simultaneously opening into one oosphere.

Minute globules of brownish color are to be seen in the cytoplasm of the oogonium from its earliest development up to zonation. In all early stages they are indefinite in number and irregular in size, but seldom larger than one of the nuclei. When the ooplasm is first differentiated it possesses a small number of these globules which are irregularly distributed. At this time and in earlier conditions there is nothing to indicate that these structures have any peculiar significance. In their color and form they suggest minute drops of some oil-like substance that has remained undissolved under all the treatment experienced by the preparation. However, at the time of zonation, and in certain later stages, there is only one such drop or globule, where there were previously several, and that one is always in the geometrical center of the oosphere, and is surrounded by a

differentiated region of cytoplasm (*figs.* 69-71, 74). Whether or not this one central globule, which is a constant feature of the oosphere from zonation until just before fertilization, is developed by a fusion of the drops previously present could not be determined with certainty. This is strongly suggested, however, by appearances like those noted in *fig.* 75, which was drawn from a young oosphere, and seems to indicate that several minute drops were fusing to form the central globule.

As the oosphere matures the central globule remains unchanged, its constancy in size being very remarkable; but the cytoplasm immediately surrounding it becomes more dense, although still shading away gradually toward the outside (*figs.* 41, 69, 71, 74). In all stages observed this globule was surrounded by the remarkable region of dense cytoplasm which differs from ordinary ooplasm in that it stains more darkly and contains fewer vacuoles and nuclei. When the whole central structure is at its maximum development it is a very conspicuous object within the oosphere (*figs.* 41, 71). There occurs also further slight differentiation of the cytoplasm in the immediate vicinity of the globule where the stain is taken more faintly or has a yellowish tint. This inner region also shows a radiate structure under the low power (*fig.* 69), but the highest magnification, such as that employed in drawing *fig.* 71 (3300 diameters), failed to demonstrate definite fibers. *Fig.* 71 shows in detail this peculiar region of the oosphere; outside is the vacuolate ooplasm, then comes the region of dense cytoplasm with few vacuoles, and finally in the center is the opaque globule immediately surrounded by a lightly stained zone. After reaching the condition of maximum development the structure rapidly loses character, disappearing entirely just before the antheridial tube discharges its contents.

A summary of the history of the central structure may be given as follows: it first appears in zonation, and reaches its maximum development when the daughter nuclei of the first mitosis pass into the oosphere; after that it rapidly degenerates, although traces of its presence sometimes persist nearly to the

time of fertilization. A body of apparently similar nature was mentioned by Wager as occurring in the oosphere of *A. candidus*, and my own as yet incomplete observations on that species indicate that the body seen in *A. candidus* and the central globule of *A. Bliti* are homologous structures, although they differ much in certain details. I find also a structure very like the one above described in the oosphere of *A. Tragopogonis* and *A. Portulacæ*, and believe that we have here an organ of the oosphere, perhaps regularly present in the whole genus *Albugo*, if not in the oospheres of other related genera. It appears with such constancy at certain important stages in the life history of the species, and passes through such a definite course of development that its presence seems to be of importance. May it not be an organ of the coenocytic oosphere?²

It should be called to mind in this connection that Dangeard noted in each of the numerous oospheres, in certain species of the Saprolegniaceæ and Peronsporeæ, a central body which appeared just before fertilization. Various interpretations have been given to such structures by different writers, early observers mistaking it for a nucleus. Dangeard supposed it to be oil, but Wager thought that Dangeard was probably mistaken, and that the structure is truly a central body such as he himself found in *A. candidus*. The descriptions and time of appearance make it seem quite possible that the body noted in the Saprolegniaceæ may be homologized with the central globule of the oosphere of *Albugo*.

It is premature to discuss the function of this body until its relations in other species have been closely studied. As described by Wager it appears to be intimately connected with the behavior of the sexual nuclei; but there appears to be no such relation in *A. Bliti*, where its function is perhaps that of an organizer in the oosphere. The body first appears when the oosphere is well defined and is most highly differentiated during the entrance of the daughter nuclei. At this time also occurs the formation of

² MR. SWINGLE expressed such views at the meeting of the Society for Plant Morphology and Physiology at Ithaca, December 1897.

a thin film of denser protoplasm which definitely bounds the oosphere. A great dynamic change occurs in the oogonium when the ooplasm and periplasm are differentiated and the zone of cytoplasm separating the two regions is formed, and in addition there is that remarkable division of the nuclei in such a manner that approximately fifty daughter nuclei are always cast into the ooplasm. Simultaneous with these activities, existing when they are at their maximum and disappearing when they cease, there is formed this peculiar structure, which is so definite in character and so constantly present that it seems to have some functional importance.

In view of the morphological character and possible physiological value of this central structure to the coenocytic oosphere the writer ventures to propose for it the name *cœnocentrum*. It is necessary here to emphasize the difference in structure between the cœnocentrum and a nucleus. The globule is distinctly not a nucleus, as it is much smaller than any nuclei that were seen, stains differently, and is structureless and unchanging. The cœnocentrum cannot be a nucleus with the globule as a nucleolus, since these masses fail to have the internal structure of a nucleus or its limiting membrane. There is no definite demarcation from the surrounding ooplasm, as is plainly shown in *figs. 41, 71, 74, 75*.

In *fig. 75* other structures are shown, the nature of which is unknown. They are small granules which lie profusely distributed in the cytoplasmic strands in all ages of the oogonium, but are particularly noticeable in the fine meshed area of the oosphere. They do not appear when stained in the ordinary way by Flemming's triple stain, but seem always present and conspicuous if stained by Heidenhain's hæmatoxylin, when they are black (*fig. 75*). A hæmatoxylin preparation when bleached and restained for a long time in safranin shows numerous round red bodies in the same position. In appearance they are a trifle longer than broad and often double, as though two were lying end to end, reminding one of large bacilli. It does not seem probable that these coalesce to form the central dot, as might

be suggested by *fig. 75*, since they have a different reaction to stain.

SIMULTANEOUS MITOSES IN THE OOGONIUM.

During the differentiation of the oosphere the nuclei in the oogonium divide once (*figs. 58-62, 65-67*), the mitosis occurring about simultaneously for all of the nuclei, cases of independent division of single nuclei never being found. Almost invariably an oogonium in the condition of zonation presents the nuclei in metaphase, or just passing into anaphase (*fig. 65*). In earlier stages, just before zonation, when the cytoplasm is massed in one or several centers, the nuclei are usually in prophase (*figs. 59-62*), but metaphase may be present even as early as the beginning of this process (*fig. 58*). It is apparent that anaphase is never reached until after zonation, and that the mitoses begin when the cytoplasm commences to collect in masses. This nuclear division always occurs when the antheridial tube is in the position shown in *fig. 59*. It would seem, *a priori*, that the differentiation of the oosphere would take place rapidly, since it consists merely in a floating out of the vacuoles and nuclei from the interior region of the oogonium, but mitosis is presumably less rapid. If changed conditions should hasten or retard the process of zonation, one would expect a variation such as does exist in the time correlations between zonation and the mitoses.

The spirem condition of the nucleus has been described, but the other stages of nuclear division have not been considered in detail. Preparatory to the formation of the spindle the nucleus elongates, its membrane being pulled out in two directions, while the chromatin collects in minute globules in the linin thread. These are at first irregular in size, but gradually become less numerous and more uniform, presumably fusing with one another until a small number of nearly spherical bodies, the chromosomes, are present in the nucleus. *Fig. 4* shows a condition with the chromatin granules and the linin network still evident, while in *fig. 5* the linin strands have almost disappeared. As the chromosomes perfect their organization they approach the equator of

the now elongated nucleus (*fig. 6*), and there appear at the poles two round bodies which lie within the nuclear membrane. These bodies stain red with Flemming's triple stain and are constantly present at this period of the prophase. Although not observed earlier they persist and become more prominent in the later stages. The spindle fibers first appear a little later at the poles of the elongated nucleus, from whence they seem to grow toward the equator. They are entirely intranuclear, and there is a distinct space between them and the nuclear membrane (*fig. 9*). The chromosomes are at first irregularly scattered throughout the equatorial region, but when the achromatic figure becomes more distinctly developed they group themselves into a nuclear plate and divide. As the chromosomes are nearly spherical and very small it was impossible to determine the manner of their division. The mature spindle rests in a clear region, distinctly inside of the nuclear membrane, with the polar bodies very definitely outlined. That these bodies must be regarded as centrosomes is evident from their constancy at certain periods of the mitosis, *e. g.*, from late prophase to late anaphase. Being intranuclear, it is not surprising that extranuclear radiations should be absent, and in fact the only radiations present are the spindle fibers. These structures seem not to have been previously described for this group of fungi.

Figs. 7, 8 show a condition very commonly seen. The nuclear membrane is prominent, the chromosomes are massed at the center, and the spindle fibers are very slightly or not at all differentiated. The dark strand shown in the lower half of *fig. 8* is probably like the less conspicuous one in a similar position in *fig. 5*. Both may be considered as the remains of a spirem thread, such as is shown in *fig. 4*. *Fig. 7* is open to a similar explanation. The difference in the shape of these two figures is noteworthy, since it is probably due to their position in the oogonium. The spindle shown in *fig. 8* lay in a strand of periplasm which supported an oosphere, similar to the strands shown in *fig. 62*. It is probable that the length of the spindle is due to the tension to which it was subjected. *Fig. 7* was from a

crowded bunch of nuclei, and could not elongate. The appearance shown in this figure might tend to support the idea that the spindle fibers are formed from the linin thread, a view entertained by Wager, but disputed by Berlese. The question presents so many difficulties that the writer does not feel warranted in expressing an opinion.

The nucleolus at the time of late prophase is sometimes small, but often quite as large as when the nucleus is in the spirem condition. It may be found throughout all stages of the mitoses. *Fig. 12* shows the splitting of the chromosomes, and *fig. 13* may be recognized as a condition immediately later; the membrane is still intact and encloses the nucleolus which lies outside of the spindle, and the centrosomes are at their maximum definiteness. It is interesting to note in passing that the few nuclei lying very near to the antheridial tube are usually nearly a full phase in advance of other oogonial nuclei in mitoses, a fact strikingly apparent when the majority of the nuclei are in metaphase.

The chromosomes, after the division of the nuclear plate, move poleward with unequal rapidity, the poles lose their acute character, and the nuclear membrane is no longer visible, the boundary of the nucleus being marked by the spindle fibers (*fig. 14*). With the loss of the membrane the whole nuclear structure assumes, and retains through later stages, the property of staining more darkly, a character particularly noticeable in the regions where the chromosomes lie (*figs. 14-18*).

The nucleolus may travel poleward with one group of chromosomes, or break into two, either at this time or earlier, thus allowing a small nucleolus to go to either pole. It is easily distinguished from the chromosomes by stain reaction and usually also by its size. At this stage there is at each apex a round body of the size and shape of the centrosomes, but scarcely distinguishable from the chromosomes except through position. Even in very late anaphase faint fibers may be seen connecting the daughter nuclei (*figs. 15, 16*). When the chromosomes reach the end of the spindle they become indistinguishably

mingled and massed (*fig. 16*), but the nucleolus often stands out very distinctly by virtue of its color and size.

After the two groups of chromosomes are sufficiently separated the spindle fibers collapse in the middle (*fig. 18*), and the daughter nuclei become distinctly organized. Each rounds off and contains a dark somewhat crescent-shaped mass of chromatin on the side that is turned away from its sister nucleus. This condition is often very noticeable in the differentiated oosphere when several daughter nuclei may be observed, each with its dark half centerward. The explanation of this condition is not far to seek. The sister nuclei lie in the periplasm with their dark half turned outward, plainly showing that the former mitotic figure lay across the line that separated the ooplasm from the periplasm. These conditions present strong evidence of the source of the nuclei in the oosphere.

Whether or not the centrosome of *Albugo* persists as a permanent organ of the cell is a question that as yet is impossible to answer. The structure so prominent at metaphase is not seen in the resting nucleus; the conditions, however, are such that it might well exist hidden among the chromatin granules and pass unnoticed. It is so small and its stain reaction so uncertain that negative evidence is valueless.

The shape of the spindle figure may be greatly modified by the conditions; for example, if the nuclei happen to be in prophase or metaphase when the centripetal rush of cytoplasm occurs the tension due to the movement of the protoplasm seems to act conjointly with the normal elongating forces, thus producing extraordinarily long spindles (*fig. 62*). On the contrary, if the nuclei reach the border of the central mass in an earlier stage of mitosis no such forces obtain. Spindles caught in the first massing of the cytoplasm are often distorted and bent like the letter *f*, owing undoubtedly to torsion caused by the vacuoles as they move outward.

MATURATION OF THE COMPOUND OOSPHERE AND OF THE
ANTHERIDIUM.

The multinucleate or compound oosphere when completely differentiated contains by actual count an average of 45-55 nuclei. These are found in various conditions of reorganization following the mitosis at zonation, and they rapidly assume the typical condition of a resting nucleus, each showing a prominent nucleolus and very faint linin network. A nuclear membrane is sharply differentiated. Presently the linin network becomes more prominent and a spirem condition is reached, very like that first observed in the oogonium. A mitosis now occurs in the oosphere affecting all of its nuclei, and is similar in all important details to that just described for the oogonium, as illustrated in *figs. 22-30*. The nuclear figure stains much more faintly than that of the previous division, the spindle appearing lighter and skeleton-like in comparison with that of the first mitoses. The only other important differences noticeable are in the more pointed anaphase and telophase figures. Compare *figs. 28-30* with *figs. 14-18*. The spindles are always long (*fig. 30*), and this fact renders it easy to detect the formation of the new membranes around the daughter nuclei by the collapsing spindle fibers. The daughter nuclei round off, pass into a resting condition, and are ready for fertilization.

While this division proceeds in the oosphere a similar mitosis occurs in the antheridium. Since the antheridial nuclei divide simultaneously with the oogonial nuclei, passing into the resting condition, and are found in mitosis when those of the oosphere divide, it is evident that they undergo two divisions. It is interesting to note that the two nuclear divisions in the antheridium and in the oogonium are similar in character and proceed simultaneously. The antheridial tube at the time of the differentiation of the oosphere lies in the periplasm, with its apex close to the bounding film of the oosphere. It now pushes into the oosphere, increasing in diameter as it advances. It takes safranin stain greedily from this time until it discharges its contents, but if the stain be thoroughly extracted in acid alcohol and the

preparation treated with gentian violet the contents become clear. The tube when fully developed is seen to contain numerous nuclei. A glance at *figs. 73, 76* will give a clear notion of this condition. It will be seen that many nuclei are massed near the tip of the tube and that others are apparently entering at the base. It is impossible to determine their number by actual count, owing to the crowded condition (*figs. 77, 85, etc.*). However, as there are about 35 nuclei originally present in an antheridium, and these divide twice, there must be altogether about 140. Of these, 20 or 30 perhaps remain in the antheridium proper, leaving a little more than 100 to pass into the tube. The antheridial tube pushes toward the center of the oosphere during the second mitosis (*fig. 70*), and arriving nearly at the center its tip swells, becoming nearly globular. In this condition the end of the tube is covered by a very thin wall which is barely visible, and yet holds within a dense mass of sperm nuclei (*fig. 77*).

When the male nuclei enter the antheridial tube they possess the characters of resting nuclei, but as they approach the tip they become oval, and later pointed at both ends, and the anterior end is seen to contain the nucleolus around which is massed a densely staining substance, probably chromatin. *Figs. 31, 32* shows nuclei from both the base and tip of the same tube, that which is illustrated in *fig. 73*. In the narrow entrance and basal portion of the tube the nuclei are necessarily arranged in single file, but as its diameter enlarges they become massed in dense groups, and the tip is so closely packed with nuclei that it reminds one forcibly of the appearance of a raspberry with its drupelets (*fig. 77*). Two sections of the same antheridial tube are shown in *figs. 77, 78*, one at the tip showing numerous nuclei surrounded by a very delicate membrane, the other near the base giving a view of the narrow nearly empty cavity and the thick wall. As the tube enlarges the protoplasm in the antheridium proper becomes more and more vacuolate, but its contents never entirely leave the structure (*figs. 80a, 86*). The film separating ooplasm from periplasm is but slightly if at all

changed by the entrance of the antheridial tube and during the maturation of the oosphere. The periplasm likewise shows no important changes. Some of its nuclei divide mitotically, but the number does not seem to increase materially. Most of them remain in a resting condition. One case was observed where every nucleus in the periplasm was undergoing mitotic division simultaneously with those of the oosphere, but this must be regarded as a very exceptional instance.

FERTILIZATION.

The conditions are now ripe for the act of fertilization. The female nuclei resulting from the mitosis in the oosphere, about 100 in number, are in resting condition. The antheridial tube is filled by an approximately equal number of male nuclei, and its tip has swollen so that the contents are separated from the ooplasm by only the thinnest of walls. The wall finally vanishes and the contents of the tube are free to mingle with the cytoplasm of the oosphere (*figs. 80, 82*). The sperm nuclei move through the ooplasm toward the female nuclei, their wake being often marked by a streak of denser cytoplasm. There is no visible cause of this movement, but as the male and female pronuclei differ in form a chemotropic influence may perhaps be safely inferred. Longitudinal sections of antheridial tubes (*figs. 80, 82*) sometimes show the nuclei pouring out, and transverse or oblique sections (*figs. 84, 85, 86, 77, 78*) corroborate this proof of a discharge of many nuclei. There is in all of these sections unmistakable evidence of a multinucleate discharge from the antheridium into a multinucleate oosphere. Sections of the antheridial tube similar to those figured, both transverse and longitudinal, are not uncommon in the writer's preparations, and many have been carefully studied. No antheridial tube was found which gave any evidence of the possibility of the discharge of but one functional sperm nucleus.

When the sperm nuclei emerge from the tube their nucleoli are in the anterior ends, and later there appears prominently in the same region a substance that stains like chromatin. As the

sperm nucleus approaches the female nucleus a faint linin network becomes visible (*figs. 33, 34, 35*). When the sex nuclei first come in contact the male is the smaller, but later they become approximately equal in size. It seems probable that the female nucleus actually decreases slightly in size during this equalization. The nuclei do not immediately fuse, though both are apparently in resting condition. All stages of fusion can be easily observed. The sexual nuclei are pressed together, the sperm nucleus first assuming a spherical form, the bounding membrane disappears at the point of contact, and there results one dumb-bell-shaped nucleus (*figs. 37, 38*). As the coalescence becomes more complete the fusion nucleus takes on a spherical form, and presents the structure of a resting nucleus. No details regarding the fate of the linin network were obtained, owing to the extreme minuteness of these structures and the increased difficulty in staining them in a manner adequate to their study (*figs. 35-40*). Fusion must be a process of extreme slowness, judging from the advance made in other structures of the oospore during its consummation.

A general view of an entire section of an oospore during the pairing of the sexual nuclei is shown in *fig. 88*. A count of the number of pairs in all of the sections of such an oospore gives an average of about 100. There seems to be a slight excess of sperm nuclei, as occasional small unpaired nuclei are found during the fusion stage; there are also several nuclei left in the antheridium proper and in the base of the tube. Sections of the oospore in which the nuclei are fusing present no trace of the antheridial tube inside of its wall, although it is easily traced through the periplasm (*fig. 91*). Judging from this condition the terminal portion of the tube must vanish immediately after giving up its contents. The portion imbedded in the periplasm becomes thickened, resembling the primitive wall; but it seems never to attain the character of the mature episporium, as is the case in so many other species of *Albugo*. Indeed, no traces of the antheridial tube were ever seen in ripe spores.

The character of the ooplasm changes when the antheridial

tube opens. As may be seen by comparing *fig. 70* with *figs. 80, 82, 84, 85* the vacuoles increase considerably in size and become irregular in form. The most striking feature of this later condition, however, is the tendency of the contents of the oosphere to break away from the periplasm (*fig. 80*), a phenomenon never met in younger stages. This indicates that changes have occurred at the boundary between the ooplasm and periplasm. Indeed, it is at this time that a true wall may be first observed around the oosphere. It will be remembered that previously the periplasm and ooplasm were separated only by the delicate film that appeared during zonation; but now for the first time a distinct wall is present around the ooplasm, and its advent seems to be correlated with the opening of the antheridial tube.

The wall occupies precisely the position of the film between the ooplasm and periplasm, and is probably formed by a further development of that structure. Its intermediate position between the ooplasm and periplasm and the apparent organic connection with both leads to the belief that it is the product of the joint action of both regions, rather than of either ooplasm or periplasm alone. Since this wall remains perfectly distinct from the walls that are formed later, it will hereafter be called the *primitive wall*. This term is used simply for convenience in this paper. Further study of related forms may establish important homologies and lead to further classification. The primitive wall is very clear and homogeneous in structure, entirely without striations, and shows great regularity of curve and thickness. From this time on the condition of the developing walls serves as an index to the age of the oospore.

A stage of somewhat frequent occurrence is shown in *fig. 87*. Judging from the presence of the primitive wall, the character of the ooplasm, and the absence of the antheridial tube, it must follow the opening of the latter, and since the nuclei are not yet paired must precede the condition shown in *fig. 88*. There are two possible explanations for this condition, consisting as it does of an oospore containing several groups of nuclei, each cluster imbedded in a mass of denser cytoplasm. Perhaps these nuclei

are gathering the cytoplasm about themselves, a phenomenon of rather frequent occurrence with sexual nuclei; or it may indicate the breaking up of the mass of nuclei and cytoplasm that was released from the antheridial tube. The latter explanation seems more probable. If it be true, a stage similar to that shown in *fig. 88* would result through a further fragmentation of these nucleated masses of denser cytoplasm.

The previous pages have dealt entirely with descriptions of the antheridial tube, the discharge of its multinucleate contents, and the subsequent fusion of sexual nuclei in pairs. For the sake of completeness, and in view of the peculiarity of the conditions and the general, if not universal, belief in a simple process of fertilization, involving only two sexual nuclei, it seems desirable to discuss the possibility of such an occurrence taking place in the oosphere, together with the events already described. A simple fertilization predicates the existence of one female nucleus in the oosphere and one male nucleus in the antheridium; these are either alone, or if with others at least different in structure and function from them. Subsequent to its final differentiation the oosphere never contains less than 40 nuclei. At a later stage both antheridium and oosphere contain about 100 nuclei, but neither contains a single nucleus differing in appearance from the others. If a uninucleated oosphere exists it must be before the oosphere is fully differentiated. A glance over the drawings shows that no such uninucleate stage is represented, nor was there ever the slightest hint of such a condition found during a most persistent search, involving hundreds of oogonia. That such could have existed and escaped observation seems very improbable. The impossibility of the central body either being or containing a nucleus has been sufficiently discussed on a previous page. The search for the single nuclei proved in vain.

The refuge left for an adherent to the idea of a simple fertilization, involving only two sexual nuclei, lies in the assumption that the nuclei of the compound oosphere (*figs. 68, 69*) have descended from a fusion nucleus, which, owing to its rapidity of development may have escaped observation in earlier conditions.

That is to say, fertilization might have occurred at a stage similar to that presented in *fig. 64* or earlier. If this were true we would expect to find the ooplasm presenting 2, 4, 8, 16, 32, 64, etc., nuclei, in stages following the division of such a fusion nucleus. As a matter of fact no such conditions were ever observed, or is there the slightest evidence that they could be present. The oosphere when first differentiated contains 40–50 nuclei, derived from the mitotic figures that line up in the manner shown in *figs. 64, 65*. This number is increased to about 100 by the mitoses in the compound oosphere (*fig. 70*), and then comes the observed act of fertilization (*fig. 85*), the discharge from the antheridial tube of a large number of sperm nuclei and the subsequent fusion of these in pairs (*fig. 88*) with the female nuclei. Previous to the act of fertilization the antheridial tube gradually fills with nuclei as it presses deeper into the ooplasm. There is of course a time when the tube contains a single nucleus, but this is when it is about one third the size finally reached, and long before it shows any indication of opening.

It is true that very early in oogenesis the dense cytoplasm in the interior of the oogonium may contain a small and very variable number of nuclei, as is shown in *figs. 61, 62, 64*. But there can be no doubt that these conditions represent part of the process of zonation, and they have been discussed in that section of this paper headed "Differentiation of the compound oosphere." It is very probable that stages similar to these might be found where there is only one nucleus left behind in the oosphere in the process of zonation, but the condition of the antheridial tube and all the further history of the oosphere show that this is not the time when an act of fertilization could possibly take place. Moreover, when only one or two nuclei are present they are *always peripheral*, which would not be expected if they resulted by the division of a fusion nucleus. Again, these scattered nuclei are always in the same condition as those near the outside of the developing oosphere, and this is almost invariably a metaphase of mitosis. This coincidence is inexplicable on the basis of their being the result of the division of a fusion nucleus,

but it follows as a necessity from the explanation offered in this paper. If they are the product of one nucleus, which has undergone three or four divisions, we have to assume not only the existence of the sex nuclei, their fusion, and the fusion nucleus, but also the resting, prophase, telophase, and anaphase conditions in the formation of 2, 4, 8, 16, and 32-celled stages. An anaphase nucleus is never seen in an oogonium except when the nuclei divide simultaneously either during the first or the second mitosis. The first anaphase always occurs when the general appearance is that shown in *fig. 67, i. e.*, when the nuclei are completely lined up and the ooplasm is well differentiated. The second appears in the oosphere after its complete differentiation (*figs. 70, 74*). It is impossible that the 50 nuclei of the oosphere can have been derived from a single hypothetical fusion nucleus.

If attention is turned to the antheridial tube it might be suggested that fertilization could take place at an early period, when the conditions are like those shown in *figs. 62, 64*. But it is only necessary to make plain the fact that the antheridial tube is always very short at this time, and invariably occupies the position shown in *figs. 62, 64*. The wall of the tube is thick, the tube rarely if ever contains nuclei, and there is not the slightest indication that it is at all ready to open. If it be assumed, however, that it does release, in some manner difficult to detect, a single nucleus that is really the male nucleus, and which fuses with the female nucleus, how can the continued growth of the tube and the development of such peculiar conditions as are shown in *figs. 68, 70, 73, 76, 77, 80, 82, 83, etc.*, be explained? Why does the tube continue to grow after functioning only to meet the difficulty of disposing of its comparatively massive body and numerous nuclei in the ooplasm? Why do its nuclei later assume a specialized form, resembling sperms (*fig. 85*)?

In considering the positive side of the argument, in favor of a multinucleate fusion, no step is left to be filled by assumption. All of the stages were seen repeatedly and the correlations are perfect. The antheridial tube opens at the culmination of a period of gradual development which has been completely traced.

After it has emptied its contents it immediately disappears. The oosphere has likewise passed through a series of remarkable but perfectly graded conditions with all the steps of development clearly shown. Coincident with the opening of the antheridial tube certain marked changes appear in the cytoplasm; the oospore wall is formed, the ooplasm immediately becomes much vacuolate where it was previously dense and uniformly constant in character. The discharge from the antheridial tube introduces into the oosphere a large number of nuclei clearly different in form from those previously there. These sperm nuclei are seen in all positions of exit from the tube, and finally become so distributed as to indicate with certainty that they approach the female nuclei.

At a stage positively older (judging by the development of the primitive wall), the oosphere is found full of fusing pairs of nuclei. That these are not nuclei dividing amitotically is proved by the number of nuclei in the oospore decreasing rather than increasing, and also by the evidence presented through detailed study.

RIPENING OF THE OOSPORE.

The process by which the oospore attains its mature condition is easily divided into three stages, which are marked by changes that take place in the oospore wall, as follows: (1) a period during which the exospore is partially constructed, and which terminates with the beginning of (2) when the primary endospore is laid down and the exospore nearly completed, and (3) a period during which the secondary endospore is formed and the oospore becomes fully ripe and ready for its winter rest.

The nuclei in the oosphere withdraw from the immediate vicinity of the primitive wall soon after fusion of the sex nuclei, or even before this act is completed, and the strands of cytoplasm toward the center thicken, leaving those of the periplasm but little more than slender supporting threads. This condition is difficult to describe but is well represented in *fig. 89*. The vacuoles have previously enlarged and the cytoplasm is more

granular than at any earlier stage. The primitive wall becomes about 0.75μ thick.

At this time a peculiar substance appears in the interstices of the ooplasm and fills the large vacuoles. It is not seen in younger stages, but is constant at this period and takes a rusty color when Flemming's triple stain is used. When the substance first appears the tint is barely perceptible, but later it assumes a brick-red color. Slightly stained sections show a film in the vacuoles, often so curled up that an edge view is obtained, thus showing that the vacuoles actually contain a stainable substance. This substance is present in stages such as those represented in *figs. 91, 92, 93, 94*.

As the primitive wall reaches maturity it becomes covered by a layer of semi-transparent substance which is penetrated by straight pores. The condition is illustrated in *fig. 92*, where a portion of this layer lies in such a position that both a surface and an edge view appear at one time. As the pores are close together and straight the structure reminds one of the hymenial surface of the *Polyporei*. Since the main substance of the layer is nearly invisible, the pores appear dark and look like papillæ or cilia growing out of the primitive wall when seen in edge-view (*fig. 92*). A condition was noted by Wager in *A. candidus*, and referred to as the "columnar condition," which from the descriptions and drawings seems similar to this. There appear somewhat later in this semi-transparent layer peculiar saucer-shaped masses of a dark substance of unknown nature (*fig. 91*). They are the first indication of the ridges so characteristic of the mature epispore. There is but little periplasm at this period, although the nuclei of the periplasm are apparently still undiminished in number. However, they stain very lightly and show only a membrane and nucleolus. They frequently mass together in bunches, but there is no evidence that they disorganize to furnish material for the exospore as is described for *A. candidus*.

To complete the endospore the saucer-shaped regions, previously mentioned (*fig. 91*), extend laterally and run together, thus forming the ridges. This gives the epispore the structure shown

in *fig. 93*. Nuclei persist much longer outside of the exospore, but they seem to be functionless, and their history was not followed further. The mature endospore consists of two layers that may be called the primary and secondary endospores according to their development. The primary endospore appears as an even layer lining the primitive wall, at first agreeing precisely in stain reaction with the gelatinous material of the vacuoles, which is still present (*fig. 93*). The primary endospore, however, soon refuses this stain and gives the reaction of cellulose with chlor-iodide of zinc. A variation in the normal sequence was found in one oospore in that the epispore was completed before any trace of endospore was to be seen.

The conspicuous feature of the third period is the laying down of the secondary endospore inside of the first. It is of cellulose, and is about equal to the primary endospore in thickness. The two layers of the fully developed endospore are clearly evident when stained, also when they cleave apart in a perfectly regular manner as they frequently do (*fig. 97*). There seems to be a pause in the laying down of cellulose when the primary endospore is completed, as is shown by the fact that perceptible changes occur in the development of the epispore after the first and before the second endospore is formed. *Fig. 94* shows the earliest trace of the secondary endospore that was seen. When the second layer of cellulose is completed the gelatinous material has completely disappeared from the vacuoles. The fact that this substance appears just before the laying down of the primary endospore, the agreement in their staining reactions while the endospore is young, and the disappearance of all of the gelatinous material simultaneously with the completion of the secondary endospore, are significant facts. They indicate that this substance is intimately connected with the formation of the cellulose walls of the spore. It appears to be transferred directly from the vacuoles to the exterior of the protoplasm, there to change to true cellulose.

Finally, various food substances accumulate in the center of the oospore, taking the form of one very large irregularly shaped

globule (*fig. 97*). Around this mass lies a zone of cytoplasm containing the fusion-nuclei in resting condition. Their number is about one hundred, so it is not probable that there has been any multiplication since fertilization. Mitotic figures have never been seen in the oospore after that act, and it is probable that the oospore persists in this condition until the following spring.

OILS AND OTHER FOODS.

A thorough discussion of this subject would involve a much more elaborate microchemical study than was undertaken. It seems desirable, however, to record a few observations since these extra-protoplasmic substances are very conspicuous at certain periods of development and their presence often introduces serious difficulties of technique. No writer has given the subject the attention that it deserves, and detailed study would doubtless yield valuable results.

There are three substances which, when present, always appear as nearly spherical globules with the general appearance of oil, and they have undoubtedly been described as such by many previous observers. Further study may show that they are not true oils, and this seems quite probable, as they do not answer to all of the microchemical tests; but in the lack of a precise knowledge of their chemical nature they may be considered here under that name. All of them are found in the serial sections cut from paraffin, that is, in material which has passed in bulk through the weak alcohols and rested in 75 per cent. alcohol for weeks or months; which has passed to the paraffin through chloroform and been cleared of paraffin by immersion for a few moments in xylol; and finally which has undergone the baths accessory to the stain employed.

The first oil is very abundant, existing in far greater quantity than either of the others, and during more stages in the development of the fungus. It is found in the young oogonium, the oosphere, and the periplasm. Diagrams showing its distribution in the important periods of development are given in the plates. *Fig. 46* presents it for an oogonium of about the age

shown in *fig. 45*. *Fig. 63* illustrates its distribution at zonation, and it should be noticed that the drops are small where the meshes of the ooplasm are fine, but large in the periplasm where the meshes are more coarse. *Fig. 72* shows the condition of an oogonium at a stage similar to *fig. 70*, a period somewhat later than zonation. The distribution is much the same because the protoplasm has changed but little. *Fig. 81*, a stage after fertilization, indicates that as the meshes of the ooplasm become coarser the globules become larger; apparently some of the globules fuse to form the larger drops. A later stage similar to *fig. 93* shows that the oil of the periplasm is nearly exhausted during the building up of the episore.

This most common oil is found in unstained sections as blackish or brown drops when the material has been killed by Flemming's fixing fluid. It is usually absent from material fixed by chrom-acetic acid, or if present is in very small quantity. If the material is allowed to remain several (six) days in the chrom-acetic fixing agent the oil appears in considerable quantity, very much as it does in Flemming's material, but of lighter straw color instead of brown. When material fails to show the oil, it seems to be really absent, because several days' immersion in 1 per cent. osmic acid solution fails to show its presence. Therefore, it seems that the osmic acid of the Flemming's fixing fluid, or the long immersion in chrom-acetic acid, so acts on the oil as to render it less easily soluble in the fluids that it meets between the killing and the time when the sections are stained. This oil when present takes a characteristic and beautiful red color from the safranin in the Flemming's triple stain combination. If it be very dark from the action of the osmic acid, it is frequently necessary to employ hydrogen peroxid to remove the blackness, in order that the best results may be obtained from the stains. It is partially on account of this oil that material killed by Flemming's fluid is not so favorable for cytological study as that fixed by the chromacetic acid.

The second oil-like substance is present only in very small quantity, possibly eight or ten small globules in an oogonium.

It is evident in both Flemming and chrom-acetic material, is stained black by Heidenhain's hæmatoxylin, but may be distinguished from the first oil by the fact that it does not take the safranin when applied in the usual way. It is figured in the plates as black dots (*figs. 51, 56, 59, 61, 64, 68, 69*); in the antheridium in *fig. 85*; in the periplasm in *fig. 80*. No difference in stain reaction was observed between this oil-like substance and the central globule of the cœnocentrum, and they may be of similar nature.

The third oil is only found in the maturing oospore, first appearing while the secondary endospore wall is developing as globules of a clear honey-yellow color in the meshes of the protoplasm (*fig. 95*). It soon accumulates at the wall, and *fig. 94* shows large drops in close contact with the forming secondary endospore, which has broken away from the primary endospore, probably owing to the impact of the knife in cutting. The drops finally become larger and more numerous, as is shown in *fig. 96*. From a study of subsequent stages it seems probable that these oil drops later break away from the wall irregularly, and unite to form the several large drops that are often found in older oospores. This results finally in the condition shown in *fig. 97*, where the entire central region is occupied by a curious irregular globular structure which stains much as the oils have stained in previous stages, but which is certainly not of fluid consistency. It probably represents reserve food material. If this structure represents a genuine oil drop in the living spore, we may have to do here with the shrunken and collapsed vesicle or membrane that encased it in life, and whose contents have been lost during the processes of technique. Its appearance would accord well with this view. From its size and the time of development in the oospore it is surely the structure described as oil by previous investigators, and is characteristic of the winter oospore.

GENERAL CONSIDERATIONS.

It seems well before closing to take a general view of the facts that have been presented, and of their relation to previous knowledge; also to point out more clearly than was possible

in earlier pages the broader significance of the phenomena described.

A mitotic division of the nuclei of fungi seems to have been first noticed by Sadeback ('83) in *Exoascus*, and has since been observed in various forms and carefully studied and described in a few papers.

The occurrence of a mitotic division in the oogonium of *Albugo* was discovered by Wager ('96) in *A. candidus*, and confirmed by Berlese ('98) in *A. Portulacæ*. Wager notes that the nuclei enlarge and divide, leaving one daughter nucleus imbedded in the central body while the other nuclei pass to the periplasm. The one division increases the nuclei from about 115 to about double that number. Berlese says the nuclei divide several times in *A. Portulacæ*, increasing the number from 30 or 40 to about 200. The account given in the present paper describes two approximately simultaneous divisions affecting all of the nuclei in both antheridium and oogonium, and these mitoses result in the formation of the sexual elements, numerous male and female nuclei. The second division is strikingly different from the first in the appearance of the nuclear elements, particularly the chromatin. This condition suggested the possibility of a reduction of chromatin, but careful study revealed no convincing evidence. The mitoses are characterized by the intranuclear formation of the spindle, the intranuclear centrosomes, the permanence of the nucleolus, and the entire absence of extranuclear radiation.

Wager described the disappearance of the nucleolus in early prophase, followed by the formation of chromosomes and then by the spindle development. He inclines toward the view that the spindle is derived from the linin. The membrane persists up to metaphase, and Wager did not follow the division further. Berlese, from observations similar to those of Wager, namely, the disappearance of the nucleolus in prophase, argues that the spindle fibers are derived from it. The behavior of the nucleolus in *A. Bliti* is different from that described by the writers mentioned, in that the structure remains apparent inside of the

nucleus through all stages of mitosis. However, since Wager studied this question only incidentally, and as Berlese gives no figures and his account is very brief, a detailed comparison of the species is impossible. Berlese reports from twelve to sixteen chromosomes, and seems to have been able to count them during the fusion of the sexual nuclei. Neither Berlese nor Wager give details of the mitosis later than prophase.

Wager reports that as near as he can estimate there are from twelve to sixteen chromosomes shown in the mitotic figures. In *A. Bliti* six are found with certainty in some anaphase nuclei, and twelve appear in some metaphase nuclei with equal certainty. It may be that when twelve are counted the chromosomes have already divided, and that they really belong to two rather than to one nucleus. However, this is not certain, and there is some evidence that makes it appear that there is a reduction in the number of chromosomes during the first mitosis, but this cannot be considered as proved.

Centrosomes were not observed in either *A. candidus* or *A. Portulacæ*, or in any other of the Phycomycetes, so far as the writer is aware; but they have been described in earlier papers for Ascomycetes (Gjurasin '93, Harper '95), and for Basidiomycetes (Wager '92, Juel '98).

With such fundamental differences as have been indicated, it is useless to attempt to establish a type of mitosis for *Albugo*, or to attempt to determine the relationship of the group through cytology. The type here described for *A. Bliti*, while presenting a few deviations from the mode of mitosis in vogue among higher plants, is in no way a departure from the forms well known among the lower types of plants and animals.

It is impossible to generalize on the facts of morphology presented in this paper, because simple processes of fertilization have been described in all forms where homology would be sought. Until the behavior of the nuclei of other species of *Albugo* is known, it is impossible to say which is the anomalous form of the genus, *A. candidus* or *A. Bliti*. From a preliminary study of *A. candidus* it can be affirmed that *A. Bliti* is in most

ways far more favorable for study; its nuclei are larger, its periplasm more abundant, its developmental stages more strongly marked, and its antheridial tube larger. *Albugo candidus*, however, has a remarkable cœnocentrum, which will be much easier to study than that of *A. Bliti*, owing to its much greater size and more pronounced stain reaction. A problem of great importance lies in a comparative study of the cœnocentra of the genus.

The characteristic massing of the cytoplasm to form a rudimentary oosphere in *A. Bliti*, differing thus from the vacuolate oosphere of *A. candidus*, is not a wider divergence than might be expected in different species; nor is the variation in the cœnocentrum more than what might be regarded as a specific difference. If such variations are found to be more marked in other species the way may be clear to trace the relationship between plants with one oosphere and those with several oospheres in each oogonium; between forms which differentiate their periplasm after the manner of *Vaucheria*, and others that follow the habit of the *Saprolegniaceæ* in a parietal rather than a central massing. It must be left to future research to make clear the relationship that must exist between the multiple fertilization illustrated by *Albugo Bliti* and simple acts of fusion between sexual elements.

It may be that cytological investigation will show remarkable variations in many respects in this genus, and establish a chain of derivative forms. The *Saprolegniaceæ* are said to range from parthenogenesis to complete sexual fertilization. Should *Albugo* prove to be similarly rich in habits the present knowledge of relationships will be much increased.

METHODS, MATERIALS, AND STAINING REACTIONS.

The material upon which this investigation was based consisted of leaves, stems, and flower clusters of *Amaranthus retroflexus* L. and *A. hybridus* L. bearing the fungus. It was collected at Syracuse, N. Y., Columbus, Ohio, and Chicago, Ill. In all cases the species seemed to be unquestionably *Albugo Bliti* Biv.

(*C. Amaranti* Schw. *C. amarantacearum* Zal.). The form described by Zalewski ('83) as a different species was not met. Oospores are very abundant on both leaves and stems, producing on the former characteristic blister-like patches that assume a blackish hue if the oospores ripen in sufficient quantity. In the stems their presence may be predicted from peculiar swellings, usually accompanied by a reddish coloration, the entire plant often being thus affected. In partially diseased plants the oospores are likely to be found in the inflorescence, which reacts much as does the stem, becoming swollen and red. The parts most favorable for study are the stems, but leaves and flowers often section more easily. In killing the material undesirable parts were cut away, and the portions apparently favorable were cut into small bits; leaves were scored, and stems and peduncles were cut in pieces about 2 to 4^{cm} long, deep incisions being made every 2^{mm} to give ready access to the killing agent. The killing with suitable solutions was apparently perfect, and was as good midway between incisions as where the solution immediately reached the tissue.

The killing agent giving the best results was chrom-acetic acid of the following formula: chromic acid 0.8 per cent., acetic acid 0.5 per cent. in water. The material was usually left in this solution from twelve to eighteen hours, then washed in five or six changes of water, allowing about two hours between changes. It was then successively transferred to 12, 25, 50, 75 per cent. alcohol, remaining about two hours in each grade, and was left in the last grade until it was practicable to imbed in paraffin. A variation in the above method, by which the material was left six days in chrom-acetic acid gave interesting results. It rendered the oil in the protoplasm much less soluble, but caused the loss of many of the details of the mitoses.

Flemming's chrom-osmic-acetic acid was employed in the same manner as the chrom-acetic acid, but was not so useful, since the sections were very much darkened by the osmic acid, thus necessitating elaborate methods of bleaching before a desirable stain could be obtained.

Other killing agents used were corrosive sublimate in saturated aqueous solution; hot corrosive-acetic-sublimate in alcohol; Carnoy's fluid; absolute alcohol; Hermann's fluid; and Merkel's fluid. Most of these gave far inferior results to that obtained by the chrom-acetic acid, and none surpassed it in effect.

In order to imbed in paraffin the material was transferred through 85, 95, and 100 per cent. alcohol to a mixture of absolute alcohol and chloroform, first of one third then of two thirds strength of chloroform. The specimens were left about two hours in each fluid, and were finally placed in pure chloroform. After the material had been left in chloroform for an hour a quantity of paraffin was added, and two hours later the material was warmed on the bath. After two hours of gentle heat it was removed to a warmer position, and later the most of the chloroform was poured off, melted paraffin substituted, and the whole kept in the bath at a temperature of about 55°. It was found advantageous during the whole process of imbedding to use very shallow dishes, as by this means most or all of the chloroform is driven off gradually by evaporation. Material was left in the bath in soft paraffin three or four hours, the paraffin being twice changed in the meantime to insure the removal of all chloroform. It was then cast in a cake, the final paraffin having a melting point of about 62°. Sections 3 to 5 μ in thickness were cut with a Jung sliding microtome and fastened to the slide with Mayer's albumen fixative.

Flemming's triple stain, used with chrom-acetic acid material, gave the best results. This stain demands the greatest attention in its use or failure is inevitable, as is well known by all who employ it. In general, the best results were attained by a bath of 30-60 min. in safranin, followed by a rinsing more or less prolonged in acid alcohol. The time here is entirely a matter of judgment, varying with the result desired; 30-90 sec. was a most usual time. After running down through the alcohols the slide was placed in saturated solution of gentian-violet for from 5-45 min. It was then rinsed in water and placed in orange

G from 5–25 sec.; a longer time may do no harm but probably 10–15 sec. is always sufficient. Wipe away excess of liquid and flood the slide twice with absolute alcohol, allowing the second lot of alcohol to remain on the slide until sufficient of the gentian-violet has been removed. The time required will depend on the material the length of time it was in the gentian-violet, and the result desired. Drain rapidly with filter paper and flood with clove oil for one minute; drain, follow by cedar oil and cover in xylol balsam. If properly stained the host cell wall should be a light violet, the chromatin of the spirem and the chromosomes blue, nucleolus and centrosomes red, and cytoplasm slightly yellowish.

Hæmatoxylin stain was used, applied after the method of Heidenhain (iron-alum 2 hrs., hæmatoxylin 12–18 hrs., followed by the slow extraction of iron alum till the proper degree is reached). This treatment gave some beautiful results in contrast with the Flemming stain, and was particularly valuable in demonstrating the achromatic portion of the nuclear figure. Hartog's nigrosin-carmin stain, as used by Wager and Berlese, was tried repeatedly on corrosive sublimate material, but the results were far inferior to those afforded by Flemming's triple or Heidenhain's hæmatoxylin stains. However, it is possible to demonstrate, even with this combination, the presence of many nuclei in the oosphere and in the antheridial tube, and to recognize the principal features of the mitotic figures. Other stains employed were Delafeld's hæmatoxylin, Biondi-Ehrlich, and cyanin-erythrosin, but they were distinctly inferior in their results.

The following stain reactions were presented in the best preparations, and were attained by the Flemming's triple stain unless otherwise stated: chromatin blue or violet, black with Heidenhain's hæmatoxylin; nucleoli red, black with Heidenhain's hæmatoxylin; centrosomes as nucleoli; spindle fibers dark blue; cytoplasm yellowish; granules, mentioned on page 163, are only seen in preparations stained with Heidenhain's hæmatoxylin, and then black.

SUMMARY.

1. The oogonium when cut off from the parent hypha contains about 300 nuclei, which enlarge and divide mitotically while the oosphere is being differentiated.

2. The oosphere is differentiated through a massing of the cytoplasm of the oogonium. By this process the nuclei, usually in stages of mitosis, together with the vacuoles, are expelled from the central region, and there results a dense and coarsely vacuolate periplasm. This condition occurs when the antheridial tube is very short.

3. There is a stage called zonation in which the nuclei, usually in metaphase, are lined up around the ooplasm, some of the spindles lying across the definite boundary that separates ooplasm from periplasm. In the telophase of this mitosis about fifty daughter nuclei are found in the ooplasm.

4. The antheridium contains at first about thirty-five nuclei which divide twice mitotically, and simultaneously with the division in the oogonium and oosphere.

5. Previous to the entrance of the antheridial tube a papilla is found projecting from the oogonium into the antheridium.

6. The antheridial tube penetrates slowly, reaching the ooplasm at the time of zonation, later entering the oosphere and appearing as a conspicuously multinucleate structure. When it opens there are discharged about one hundred male nuclei which fuse with the female nuclei in pairs.

7. The sexual nuclei differ in form; the sperm being elongated and the egg spherical.

8. A peculiar central body, the cœnocentrum, develops as the oosphere matures and disappears before fertilization. Its function is unknown. There is some evidence of its being a dynamic center of the compound oosphere.

9. The mitoses are alike in the oogonium and antheridium. The spindle is intra-nuclear and there are no extra-nuclear radiations. The centrosomes are very prominent at metaphase, and are intra-nuclear. They could not be distinguished in the

resting nucleus. The nuclear membrane persists until after metaphase and the nucleolus is present throughout the division.

10. The primitive wall of the oospore first appears when the antheridial tube opens. Later the epispore is laid down upon it by the periplasm.

11. Two endospores are formed by the ooplasm after the development in the vacuoles of a peculiar substance which disappears as the endospores reach maturity.

12. After the complete encasement of the oospore it becomes rapidly filled with food-stuffs. A large central oil-like drop is present during the winter condition,

13. The fusion nuclei pass the winter in the resting condition without further perceptible change.

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EXPLANATION OF PLATES XI-XV.

All figures are from material killed in chrom-acetic acid and stained with Flemming's triple stain, unless otherwise indicated. The figures were sketched with an Abbé camera, using the following combinations of lenses: Zeiss 2^{mm} ap. 1.30, with compensating ocular 18 and 12; also Bausch & Lomb 1 $\frac{1}{2}$, with ocular no. 4. These combinations give respectively the following magnifications when projected to the table level: 3300, 2200, and 1500 diameters. *Plate XI* was not reduced in reproduction. All other plates are three fourths of the original scale.

PLATE XI.

All drawings represent a magnification of 3300 diameters, and were studied with Zeiss lenses.

FIG. 1. Resting nucleus in mycelium, linin faint, nucleolus prominent.

FIG. 2. Nucleus in flowing cytoplasm near entrance of oogonium.

FIG. 3. Nucleus in antheridium, spirem stage, membrane faint.

FIG. 4. Prophase: small drops accumulated on the linin network, the thread itself thinner, nucleus becoming spindle-shaped, no centrosome visible.

FIG. 5. A stage somewhat later, linin threads have almost entirely disappeared, chromatin grains scattered through nucleus, nucleolus to the right, and remains of a linin strand to the left.

FIG. 6. Nucleus more spindle-shaped, globules arranged in the equatorial plate, at the poles round bodies about equal to the chromatin dots in size, no spindle fibers, the definite line bounding the whole of the nuclear membrane.

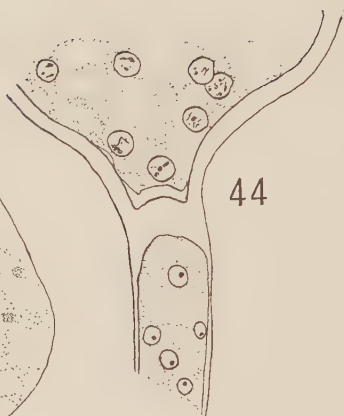




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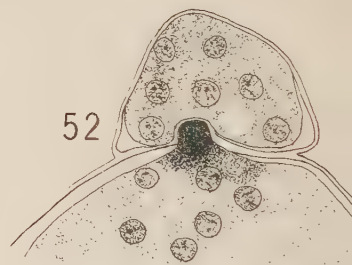
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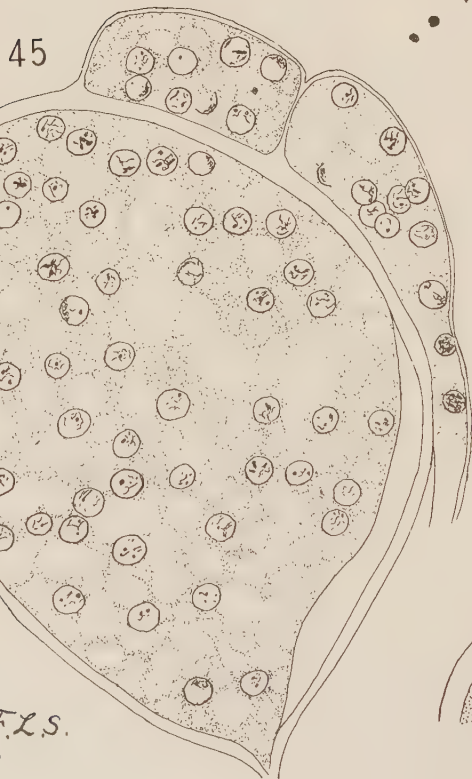
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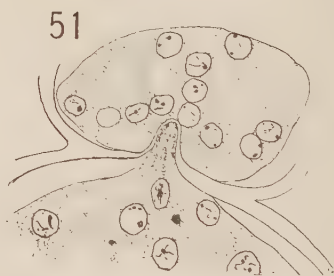
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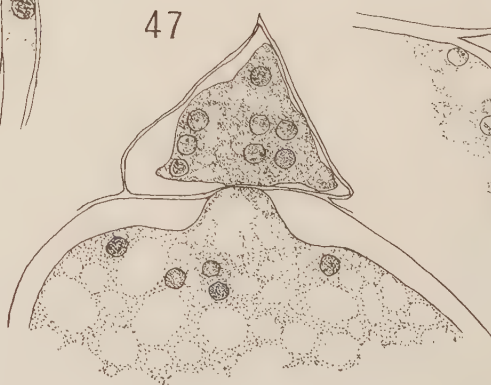
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F.L.S.





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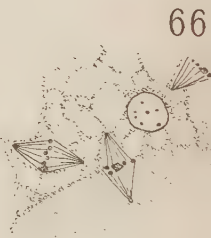
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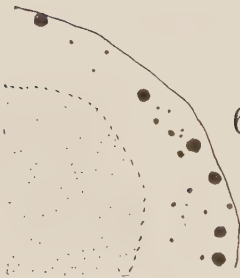
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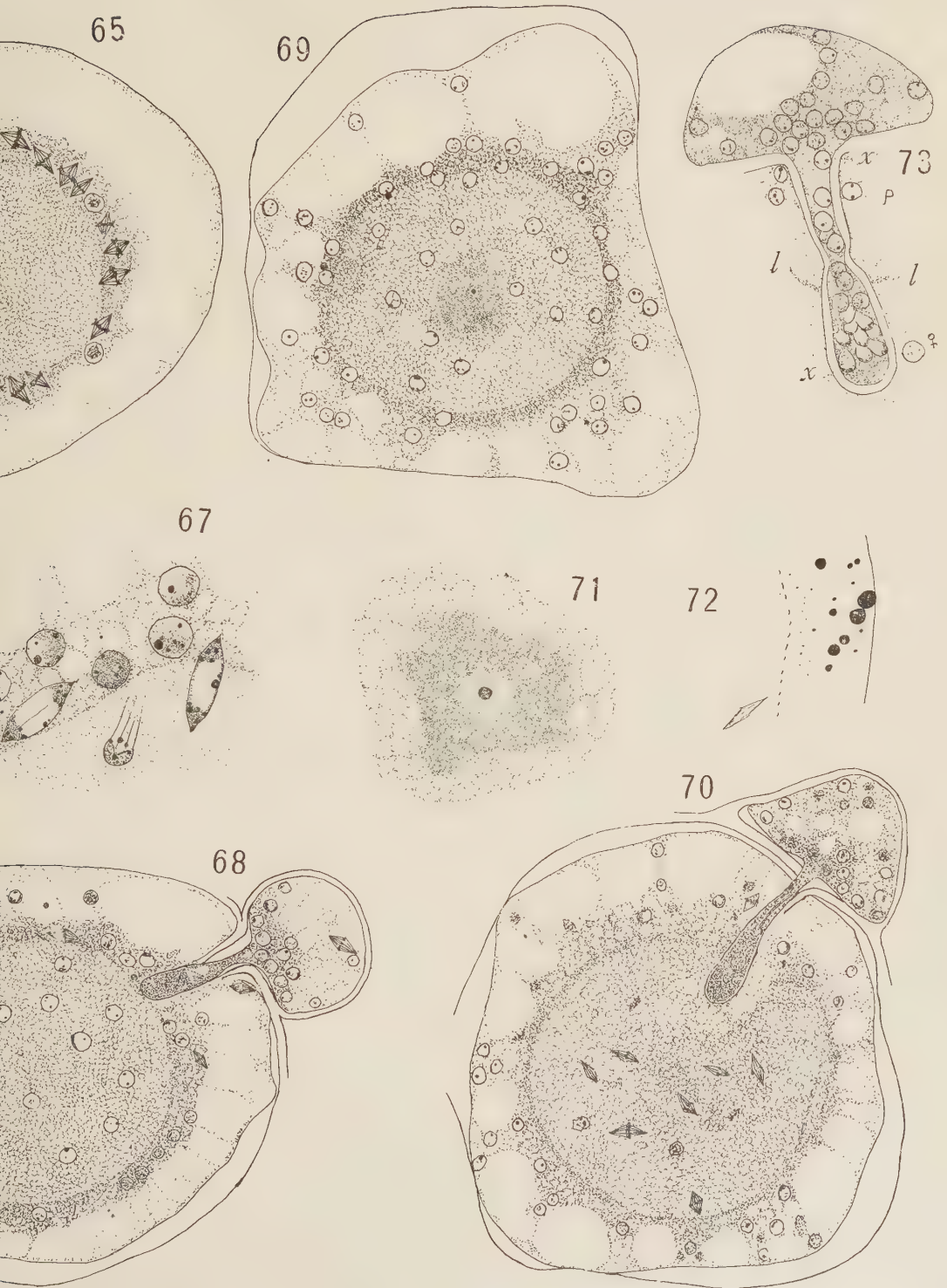


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76

b

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b

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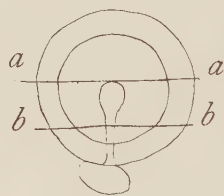
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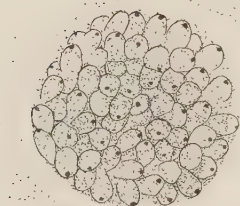
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a

b

b

77



80 a

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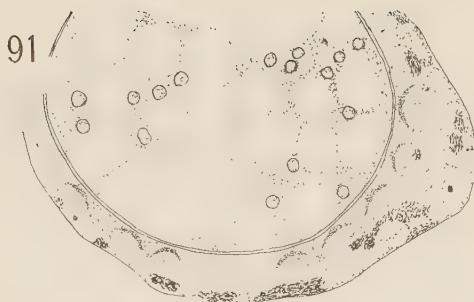
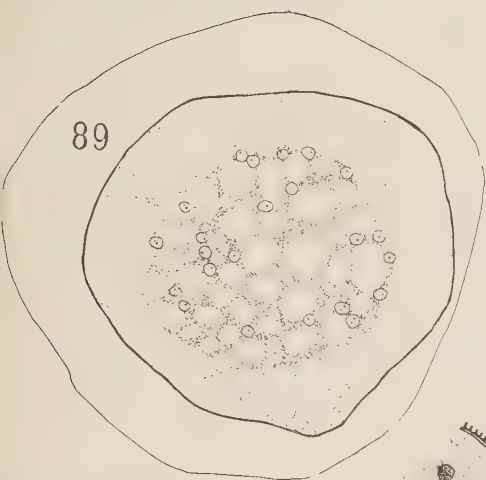


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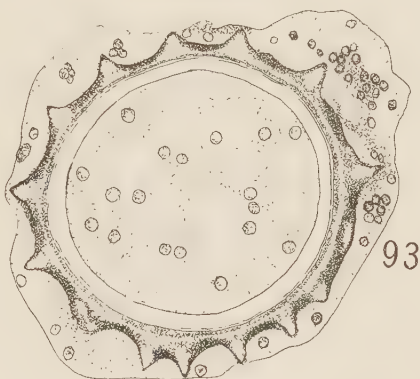
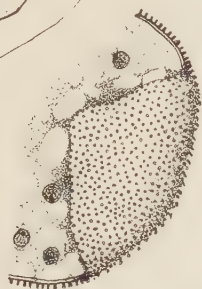
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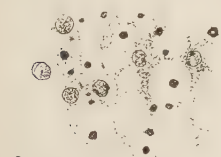


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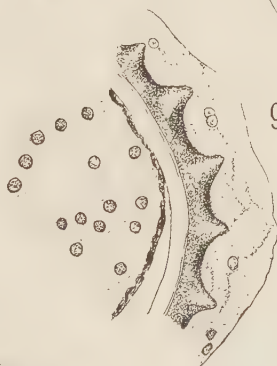


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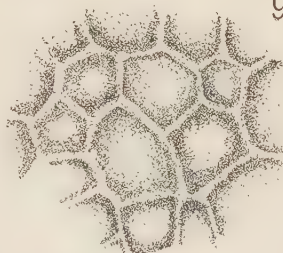


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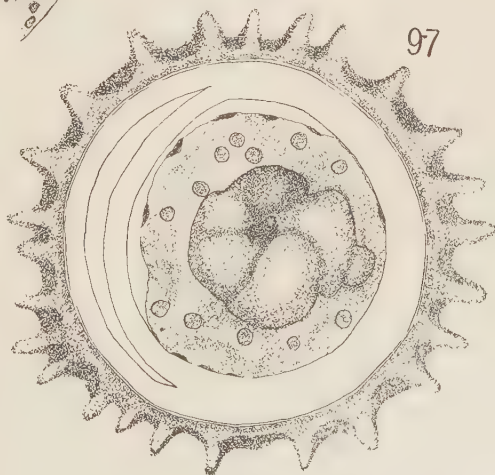
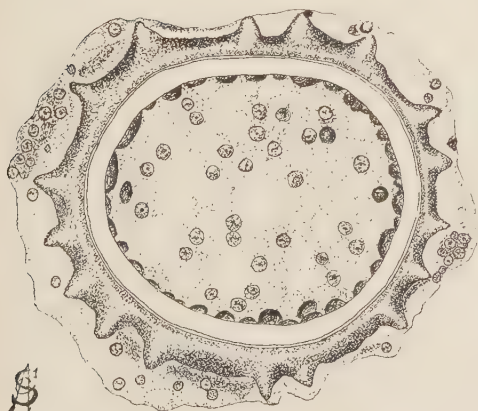


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FIG. 7. Similar to *fig. 5*. The longitudinal lines probably chromatin which has not reached the equator, nucleolus at the left. This nucleus was crowded by a mass of others, hence its short form.

FIG. 8. Similar to *fig. 7*. Fibers visible toward the poles. This spindle was formed in one of the strands supporting the forming oogonium, and was consequently much elongated by tension. It is from the oogonium shown in *fig. 62*.

FIG. 9. Chromosomes at the equator, spindle fibers very apparent at the poles, but not visible at the equator, nucleolus to left, membrane intact and inclosing the spindle.

FIG. 10. From oogonium shown in *fig. 60*. Cross section of a spindle, twelve chromosomes apparent (stained by hæmatoxylin from Flemming's material).

FIG. 11. Spindle mature, chromosomes closely grouped at the equator, centrosomes prominent, spindle brilliant and clear, nuclear membrane present but poorly stained (*fig. 13*).

FIG. 12. Chromosomes splitting, membrane visible with nucleolus enclosed.

FIG. 13. Nucleolus large, nuclear membrane very definite, daughter chromosomes ready to separate. Only those in the highest focus are shown, several others being found at a deeper focus. The stain was particularly to show the membrane, and was not suitable for centrosomes.

FIG. 14. Anaphase: chromosomes separating, nucleus lying about midway, centrosomes still visible, whole nucleus staining dark and membrane indistinguishable from spindle fibers.

FIG. 15. Chromosomes nearing the poles, centrosomes not distinguished from them, nucleolus midway, slight traces of spindle fibers stretching across the middle space, cytoplasm in the ends of the nucleus stains darker than that of the central area.

FIG. 16. Whole spindle elongated, chromosomes massing together at the poles.

FIG. 17. Similar to *fig. 16*, but in a crowded position. Compare with *fig. 7*.

FIGS. 18 and 19 are from the periplasm of an oogonium of about the age shown in *fig. 67*, slightly younger than shown in *fig. 68*.

FIG. 18. Spindle fibers collapsing in the middle leading to the separation of the daughter nuclei, the fibers constituting the origin of the membrane of the daughter nucleus.

FIG. 19. A young daughter nucleus, nucleolus, membrane, and chromosomes.

FIG. 20. Same as *fig. 19* in resting stage, nucleolus prominent, linin faint.

FIGS. 21 and 22 are from the same oosphere, and in the condition shown in *figs. 68-70*.

FIG. 21. Same as *fig. 20* but passing into the spirem stage.

FIG. 22. Nucleus elongating preparatory to division. Compare *fig. 4*.

FIG. 23. Breaking of skein into chromosomes, centrosomes apparent.

FIG. 24. Spindle forming inside of nuclear membrane, nucleolus lying outside of the spindle.

FIG. 25. From an oospore of the condition shown in *fig. 70*. Spindle lying completely inside of the nuclear membrane, chromosomes grouping at the equator, and centrosomes well defined.

FIG. 26. Metaphase: chromosomes splitting, membrane, centrosomes, and fibers clear. Fibers apparently of about the same number as the chromosomes.

FIG. 27. Daughter chromosomes ready to leave the equator.

FIG. 28. Anaphase: chromosomes well separated, centrosomes visible, spindle fibers crossing the middle space, chromosomes six in number. From same oospore as *figs 24* and *27*. Compare with *fig. 16*.

FIG. 29. Later anaphase: chromosomes near the poles, area in which they rest darker stained than central portion.

FIG. 30. Similar to *fig. 18*. Spindle fibers collapsed and daughter nuclei ready to separate. From same oospore as *fig. 29*.

FIG. 31. A male nucleus from the entrance of antheridial tube. This nucleus is the same as the one marked X in *fig. 73*.

FIG. 32. A nucleus (sperm) in the tip of the same tube that contained that shown in *fig. 31*, also marked X. Wall of the antheridial tube, dense cytoplasm surrounding nuclei, sperm with nucleolus and mass of chromatin at anterior end.

FIG. 33. Tube open, elongated and pointed sperms escaping, showing a very faint linin network, one female nucleus shown. This is part of the drawing shown in *fig. 85*.

FIG. 34. A sperm approaching a female nucleus, linin more prominent than in *fig. 33*.

FIG. 35. A sperm in contact with a female nucleus, becoming more nearly round, and its linin still more prominent.

FIGS. 36 to 40. Various stages of fusion, from oospores of the general appearance represented in *fig. 90*. During fusion the whole nucleus becomes more darkly stained with gentian-violet.

FIG. 41. Cœnocentrum with globule at its center, female nucleus near by, ordinary vacuoles of the oosphere near the margin.

PLATE XII.

Magnification in all figures 1500 diameters.

FIG. 42. A portion of mycelium, showing nuclei with prominent nucleoli.

FIG. 43. Nuclei and cytoplasm flowing into a developing oogonium, nuclei and vacuoles elongated and angular, nuclei too darkly stained to show structure.

FIG. 44. The septum below the oogonium, a portion of mycelium and posterior end of oogonium, oogonial nuclei assuming the spirem stage, mycelial nuclei still in resting condition with prominent nucleoli but enlarged.

FIG. 45. Oogonium and antheridium, nuclei of both in the spirem stage.

FIG. 46. Shows location of oil drops in an oogonium of the condition presented in *fig. 45*. They were all drawn at one focus; a slight change of focus would have brought vastly more into view.

FIG. 47. Showing adhesion of the oogonial *Hautschicht* to its wall in the neighborhood of the antheridium, nuclei overstained.

FIG. 48. Adhesion as in *fig. 47*. Oogonial wall partly and irregularly corroded away on the side toward the oogonium.

FIGS. 49-55. Stages in the perforation of the wall preparatory to the entrance of the antheridial tube.

FIG. 49. Optical section of papilla, wall partly corroded away and bulging toward the antheridium. The dense protoplasm represented in black was stained a deep red by the safranin.

FIG. 50. Sectional view of a condition slightly older than *fig. 49*. The shaded portion of the separating wall took the stain differently from the rest, and was apparently in the last stages of dissolution.

FIG. 51. A papilla of different shape in a stage similar to the last.

FIG. 52. Optical section, stain as in *fig. 49*.

FIG. 53. A papilla becoming bubble-like, walls very thin, slightly shrunken as though due to imperfect killing, watery vacuoles apparent, their contents staining homogeneously with the gentian violet.

FIG. 54. Bubble-like papilla expanding irregularly in all directions, contents highly vacuolate, wall extremely thin.

FIG. 55. Wall almost perforated, but showing no marked bulging in either direction, *o*, oogonial, *a*, antheridial side.

FIGS. 56, 57. Young antheridial tubes with a cellulose wall and surrounded by a dense sheath of the protoplasm of the oogonium.

FIG. 58. Protoplasm beginning to collect in masses, nuclei approaching metaphase, some shown in polar view (preparation from material fixed in Flemming's agent and stained by hæmatoxylin).

FIG. 59. An intercalary oogonium, slightly older condition than *fig. 58*, cytoplasm distinctly in masses, nuclei in early prophase, antheridial tube just entering bearing no nuclei, a haustorium at extreme right.

PLATE XIII.

Magnification in *figs. 60, 61, 62, 63, 64, 65, 68, 69, 70, 72*, 1500 diameters; in *figs. 66, 67, 71*, 3300 diameters; in *fig. 73*, 2200 diameters.

FIG. 60. Nuclei in metaphase, protoplasm massed in a few centers, spindles very clear and brilliant (stain hæmatoxylin from material fixed in Flemming's agent).

FIG. 61. Nuclei in late prophase, the protoplasmic masses coalesced to form one, vacuoles mark the juncture last made, a few nuclei not yet floated out.

FIG. 62. Nuclei approaching metaphase, spindles much elongated, all not yet extruded from the central region, mitosis in the antheridium, antheridial tube shown at its typical position for this stage.

FIG. 63. Diagram of disposition of oil drops in an oogonium of the condition shown in *fig. 65* (zonation).

FIG. 64. Nuclei nearly at metaphase, zonation almost complete, oil-like drop to the right in ooplasm, antheridial tube present showing no nuclei.

FIG. 65. Zonation: nuclei near metaphase, ooplasm sharply differentiated, many spindles lying directly across the boundary between ooplasm and periplasm (stained by hæmatoxylin from Flemming material). See also *fig. 64*.

FIG. 66. Shows spindle in metaphase lying across the film between ooplasm and periplasm.

FIG. 67. Nucleus in anaphase directly across the boundary film of the ooplasm, also half of a late anaphase cut diagonally.

FIGS. 68, 69. Consecutive sections of an oogonium just after the division of the nuclei, showing the position and shape of the antheridial tube. *Fig. 69* shows the cœnocentrum which had just passed its maximum development (see *fig. 71*).

FIG. 70. Antheridial tube and differentiating oosphere, oosphere nuclei in mitosis. *Fig. 74* was taken from an adjacent section of the same oogonium and shows the cœnocentrum.

FIG. 71. Completely developed cœnocentrum, the central globule surrounded by three regions of differentiated protoplasm. From a stage of zonation where the nuclei were in anaphase (as in *fig. 67*) and the daughter nuclei were about to pass into the ooplasm.

FIG. 72. Diagram of distribution of oil at the stage shown in *fig. 70*.

FIG. 73. Antheridium and tube of the age shown in *fig. 70*, showing multinucleate contents; *L*, film; *♀*, one of the female nuclei; *p*, periplasm; *x, x*, nuclei, represented also in *Plate XI, figs. 31, 32*.

PLATE XIV.

Magnification in *figs.* 74, 80, 82, 87, 88, 1500 diameters; *fig.* 76, 2200 diameters; *figs.* 75, 77, 78, 84, 85, 86, 3300 diameters.

FIG. 74. Cœnocentrum and differentiated oosphere with dividing nuclei. The broken empty portion is where the protoplasm shrunk away from the antheridial tube. Section adjacent to that shown in *fig.* 70.

FIG. 75. Cœnocentrum, in an oogonium, at anaphase period of zonation, consisting of three small globules evidently fusing, surrounded by a region of denser protoplasm, granules resembling small oil drops scattered through the ooplasm.

FIG. 76. Similar to *fig.* 73 but somewhat older, antheridial tube showing many nuclei, slightly torn near tip, antheridium becoming vacuolate; *p*, periplasm; *b*, film; *♀*, female nucleus.

FIG. 76 *a*. Portion of the tip of the same tube, found in an adjacent section.

FIG. 77. View of the end of an unopened antheridial tube, wall not apparent, sperms very numerous and crowded, each showing a dark nucleolus, ooplasm slightly shrunken away from the tube.

FIG. 78. Section near the base of the same antheridial tube, showing very thick wall in contrast with the extremely thin film covering the apex.

FIG. 79. Diagram to show position of preceding sections. *Fig.* 77 was from a section just above the line *aa*; *fig.* 78 from one just below the line *bb*. One intermediate section was not drawn.

FIGS. 80, 80*a*. Adjacent and approximately longitudinal sections of the opening antheridial tube of the same oogonium, showing the primitive wall and the increased vacuolation of the ooplasm which frequently shrinks away from the primitive wall.

FIG. 81. Diagram of the distribution of oil in an oogonium of the age shown in *fig.* 80.

FIG. 82. Antheridial tube discharging sperms, antheridium shown above, primitive wall very young, ooplasm becoming vacuolate, antheridial tube cut slightly oblique so that its base is in an adjacent section.

FIG. 83. Diagram to show relation of sections presented in *figs.* 84, 85, 86: *fig.* 84 was cut from above the line *aa*, and tangential to the tip of the opening tube; *fig.* 85 from between the lines; *fig.* 86 from just below the line *bb*.

FIG. 84 (see *fig.* 83). Sperm nuclei leaving the antheridial tube and approaching the female nuclei.

FIG. 85 (see *fig.* 83). Mass of sperms escaping from tube. The tube may be traced to the left as a mass of darkly stained structureless protoplasm.

The sperms in a mass appear dark but individually are hyaline except at anterior end which bears the nucleolus. See also *fig. 33, Plate XI*.

FIG. 86 (see *fig. 83*). The base of the antheridial tube filled with dark staining cytoplasm and few nuclei.

FIG. 87. Just after discharge from antheridial tube, periplasm as in *fig. 82*, several small masses of nuclei apparently both male and female surrounded by denser cytoplasm (these masses seem to arise by the breaking apart of the contents of the antheridial tube and soon disappear), primitive wall well developed, antheridium above, ooplasm coarsely and irregularly vacuolate.

FIG. 88. Nuclei fusing in pairs, primitive wall distinctly thickened. See *Plate XI, figs. 36-40*.

PLATE XV.

All figures magnified 1500 diameters.

FIG. 89. Protoplasm collecting in dense network toward the center of the oospore, leaving light peripheral strands where the endospore is soon to appear.

FIG. 90. Shows the remains of the antheridial tube in the periplasm, with no trace of its former presence in the ooplasm. The oospore is of the age shown in *fig. 88*.

FIG. 91. Primitive wall mature, exospore forming, now consisting of a porous semi-transparent mass with imbedded disks which are to form the ridges, vacuoles in oospore filled with gelatinous substance, nuclei of protoplasm overstained.

FIG. 92. Somewhat younger than the last figure, showing structure of exospore more clearly, presents edge view and also a fragment bent back and giving a surface view, gelatinous substance in vacuoles.

FIG. 93. Exospore nearly formed, primary endospore complete, vacuoles still containing gelatinous substance.

FIG. 94. Rudimentary secondary endospore, gelatinous substance in vacuoles.

FIG. 95. Oil forming in oospore.

FIG. 96. Spore walls complete, including double endospore, vacuoles with no gelatinous substance, oil accumulating in large drops on the endospore walls.

FIG. 97. Winter conditions of exospore, the ridges higher than in previous stages, large central oil-like mass, nuclei in sporoplasm. Section not directly through middle so that the endospore appears thicker than it really is.

FIG. 98. Exterior of ripe oospore.

